

CLAIMS

We claim:

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1. A method of producing a protein with an increased activity or stability, comprising:

replacing an arginine residue capable of being ADP-ribosylated with a tryptophan residue or a phenylalanine residue in a position of an amino acid

10 sequence of the protein, thereby producing the protein with increased activity or stability.

2. The method of claim 1, wherein the protein has an increased antimicrobial activity.

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3. The method of claim 2, wherein the antimicrobial activity comprises chemotaxis of T cells, neutrophil recruitment, or cytokine release.

4. The method of claim 3, wherein the cytokine release comprises 20 interleukin-8 release.

5. The method of claim 2, wherein the protein is a defensin.

6. The method of claim 5, wherein the defensin is an alpha defensin.

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7. The method of claim 2, wherein the arginine residue is substituted in the amino acid sequence of the protein with a tryptophan residue.

30 8. The method of claim 2, wherein the arginine residue is substituted in the amino acid sequence of the protein with a phenylalanine residue.

9. The method of claim 2, wherein the activity is increased as compared to a polypeptide having an arginine residue in the position of the amino acid sequence of the protein.
- 5 10. The method of claim 2, wherein the stability is increased as compared to a polypeptide having an arginine residue in the position of the amino acid sequence of the protein.
- 10 11. The method of claim 2, wherein the increased activity or stability is a 100% increase, or a 100% decrease, as compared to a control polypeptide.
12. The method of claim 2, wherein the increased activity or stability is a 50% increase, or a 50% decrease, as compared to a control polypeptide.
- 15 13. A method of determining if a protein can be stabilized, comprising:
determining if an arginine residue in the protein is capable of being ADP-ribosylated.
- 20 14. The method of claim 13, wherein the protein has an antimicrobial activity when administered to a subject.
15. The method of claim 14, wherein determining if an arginine residue in the protein is capable of being ADP-ribosylated comprises:
25 contacting the protein with an ADP-ribosyltransferase capable of ADP-ribosylating the arginine residue;
measuring an electrophoretic mobility of the protein that was in contact with the ADP-ribosyltransferase; and
30 comparing the electrophoretic mobility of the protein to an electrophoretic mobility of a first control, wherein a decrease in electrophoretic mobility of the protein, compared to the first control, is an indication that the protein is ADP-ribosylated, thereby determining if the arginine residue in the protein is capable of being ADP-ribosylated.

16. The method of claim 14, wherein the antimicrobial activity comprises chemotaxis of T cells, neutrophil recruitment or cytokine release.

5 17. The method of claim 14, wherein the protein is a defensin.

18. The method of claim 17, wherein the defensin is an alpha defensin.

19. A composition comprising, a polypeptide comprising an amino acid sequence wherein at least one arginine residue capable of being ADP-ribosylated is substituted with a tryptophan or a phenylalanine residue, wherein the substitution increases the activity or stability of the polypeptide.

20. The composition of claim 21, wherein the polypeptide has an
15 antimicrobial activity.

21. The composition of claim 20, wherein the arginine residue is substituted with a tryptophan residue.

20 22. The composition of claim 20, wherein the arginine residue is substituted with a phenylalanine residue.

23. The composition of claim 20, wherein the antimicrobial activity comprises chemotaxis of T cells, neutrophil recruitment, or cytokine release.

25 24. The composition of claim 20, wherein the protein is a defensin.

25. The composition of claim 24, wherein the defensin is an alpha defensin.

30 26. A pharmaceutical composition comprising a therapeutically effective amount of a defensin comprising at least one arginine residue that is substituted by a tryptophan or a phenylalanine residue.

27. The pharmaceutical composition of claim 26, wherein the defensin has antimicrobial activity.

5 28. The pharmaceutical composition of claim 27, wherein the antimicrobial activity comprises chemotaxis of T cells, neutrophil recruitment or cytokine release.

10 29. A method of increasing the activity or stability of a defensin polypeptide comprising an arginine residue capable of being ADP-ribosylated, comprising substituting the arginine residue with a tryptophan or a phenylalanine, thereby increasing the activity or the stability of the defensin polypeptide.

15 30. The method of claim 29, wherein the defensin polypeptide is an alpha defensin.

31. The method of claim 29, wherein the activity is an antimicrobial activity.

32. The method of claim 31, wherein the antimicrobial activity comprises T cell chemotaxis, neutrophil recruitment, or cytokine release.

20 33. A method of increasing an immune response in a subject, comprising administering to the subject a therapeutically effective amount of a defensin polypeptide comprising an amino acid substitution, wherein the amino acid substitution is replacement of an arginine capable of being ribosylated with a tryptophan or a phenylalanine, thereby modifying the immune response in the subject.

25 34. The method of claim 33, wherein the immune response comprises T cell chemotaxis, neutrophil recruitment, or cytokine release.

30 35. The method of claim 33, wherein the subject has an immune disorder.